




Draft Genome Sequence of *Bacillus licheniformis* Strain UASWS1606, a Plant Biostimulant for Agriculture

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ABSTRACT *Bacillus licheniformis* is a well-known industrial bacterium. New strains show interesting properties of biostimulants and biological control agents for agriculture. Here, we report the draft genome sequence, obtained with an Illumina MiniSeq system, of strain UASWS1606 of the bacterium *Bacillus licheniformis*, which is being developed as an agricultural biostimulant.

Bacillus licheniformis is a Gram-positive, endospore-forming, saprophytic motile bacterium that commonly occurs in plants, soils (1), or even birds' feathers (2). Current taxonomy shows that it is closely related to *Bacillus subtilis* (3). It is a well-known bacterium used for its enzymes and antibiotics in a wide range of industries (4, 5), and some strains have already demonstrated interesting features for agronomic applications, such as imparting increased resistance to salt-alkaline stress (6), presenting endophytic behavior with biocontrol properties (7), and promoting plant growth and demonstrating fungus-antagonizing properties (8, 9). *Bacillus licheniformis* UASWS1606 was isolated from an agricultural clay loam soil in Presinge, Geneva, Switzerland (10), and initially identified as *Bacillus licheniformis* by 16S rRNA gene Sanger sequencing.

DNA was extracted with a modified cetyltrimethylammonium bromide (CTAB) protocol (11) from a culture grown exponentially overnight in Luria-Bertani broth at 25°C from a single colony. A sequencing library was built with the TruSeq Nano DNA library preparation kit (Illumina, USA). Whole-genome sequencing was performed using a MiniSeq high-output kit within one Illumina MiniSeq run with a 2 × 151-bp paired-end read length, which produced 6,409,906 reads, resulting in 220× genome coverage. The overall quality metrics of the reads were assessed with FastQC v0.11.5 (12). Genome assembly was performed with the SPAdes genome assembler v3.13.0 (13) with a setting of "paired-end assembly, careful mode," yielding 33 contigs (≥200 bp), ordered with BioEdit v7.0.5 (14), and analyzed with QUAST v4.6.3 (15) with the setting of "QUAST: skip contigs shorter than 200 bp." The total genome length is 4,370,390 bp, with a GC content of 45.69% and an N_{50} value of 1,154,533 bp. Automated gene annotation carried out with the NCBI Prokaryotic Genome Annotation Pipeline (PGAP) v4.1 (16) identified 4,396 coding sequences and 83 RNA genes, while RAST v2.0 (17), using the classic RAST annotation scheme, detected 4,677 coding sequences and 81 RNA genes. PlasmidFinder v1.3 (18) and plasmidSPAdes, both using default settings, did not detect any plasmids. RAST v2.0 (17) did not find any complete transposons or phages integrated. NCBI BLAST (19) showed that the whole 16S rRNA gene (1,456 bp) shared 99.93% identity with 7 *Bacillus paralicheniformis* strains and then 99.86% identity with 13 *Bacillus licheniformis* and 2 *Bacillus paralicheniformis* strains. According to the NCBI SRA Taxonomy Analysis Tool (STAT), based on raw sequencing read analysis, *Bacillus licheniformis* UASWS1606 shares 56.5% of its genome with *Bacillus licheniformis*, 18.7%

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with *Bacillus paralicheniformis*, and 17.6% with *Bacillus haynesii*. The annotation confirmed the absence of toxins and superantigens, as well as virulence and disease genes, allowing this bacterium to be considered for agronomic and environmental uses. Regarding its agronomic application, four genes of the auxin biosynthesis pathway and many protein-coding genes involved in the biocontrol process, such as genes for transporters, plant cell lytic enzymes, siderophores, and other secondary metabolites, are present. Like *Bacillus licheniformis* strain CKA1 (20), the presence of 15 genes related to phosphorus metabolism, including the Pho operon, suggests a strong ability to solubilize phosphate. Phenotypic profiling confirmed auxin synthesis and phosphate solubilization. Annotation also revealed 22 genes linked to nitrogen metabolism, some of which may increase plant growth rates and biomass production.

Data availability. This whole-genome shotgun project was deposited in DDBJ/EMBL/GenBank under the accession number [JAAOWI000000000](https://www.ncbi.nlm.nih.gov/nuclseq/JAAOWI000000000). The version described in this paper is the first version, [JAAOWI010000000](https://www.ncbi.nlm.nih.gov/nuclseq/JAAOWI010000000). Raw sequencing data sets have been deposited in the NCBI Sequence Read Archive (SRA) database under the accession number [SRX7906581](https://www.ncbi.nlm.nih.gov/sra/SRX7906581).

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REFERENCES

- Veith B, Herzberg C, Steckel S, Feesche J, Maurer KH, Ehrenreich P, Bäumer S, Henne A, Liesegang H, Merkl R, Ehrenreich A, Gottschalk G. 2004. The complete genome sequence of *Bacillus licheniformis* DSM13, an organism with great industrial potential. *J Mol Microbiol Biotechnol* 7:204–211. <https://doi.org/10.1159/000079829>.
- Williams CM, Richter CS, Mackenzie JM, Shih JC. 1990. Isolation, identification, and characterization of a feather-degrading bacterium. *Appl Environ Microbiol* 56:1509–1515. <https://doi.org/10.1128/AEM.56.6.1509-1515.1990>.
- Rey MW, Ramaiya P, Nelson BA, Brody-Karpin SD, Zaretsky EJ, Tang M, Lopez de Leon A, Xiang H, Gusti V, Clausen IG, Olsen PB, Rasmussen MD, Andersen JT, Jørgensen PL, Larsen TS, Sorokin A, Bolotin A, Lapidus A, Galleron N, Ehrlich SD, Berka RM. 2004. Complete genome sequence of the industrial bacterium *Bacillus licheniformis* and comparisons with closely related *Bacillus* species. *Genome Biol* 5:R77. <https://doi.org/10.1186/gb-2004-5-10-r77>.
- Eveleigh DE. 1981. The microbial production of industrial chemicals. *Sci Am* 245:154–178. <https://doi.org/10.1038/scientificamerican0981-154>.
- Erickson RJ. 1976. Industrial applications of the bacilli: a review and prospectus, p 406–419. In Schlessinger D (ed), *Microbiology*. American Society for Microbiology, Washington, DC.
- Zhou C, Zhu L, Xie Y, Li F, Xiao X, Ma Z, Wang J. 2017. *Bacillus licheniformis* SA03 confers increased saline-alkaline tolerance in *Chrysanthemum* plants by induction of abscisic acid accumulation. *Front Plant Sci* 8:1143. <https://doi.org/10.3389/fpls.2017.01143>.
- Nigris S, Baldan E, Tondello A, Zanella F, Vitulo N, Favaro G, Guidolin V, Bordin N, Telatin A, Barizza E, Marcato S, Zottini M, Squartini A, Valle G, Baldan B. 2018. Biocontrol traits of *Bacillus licheniformis* GL174, a culturable endophyte of *Vitis vinifera* cv. Glera. *BMC Microbiol* 18:133. <https://doi.org/10.1186/s12866-018-1306-5>.
- Sukkasem P, Kurniawan A, Kao T-C, Chuang H-W. 2018. A multifaceted rhizobacterium *Bacillus licheniformis* functions as a fungal antagonist and a promoter of plant growth and abiotic stress tolerance. *Environ Exp Bot* 155:541–551. <https://doi.org/10.1016/j.envexpbot.2018.08.005>.
- Neyra C, Atkinson LA, Olubayi O, Sadasivan L, Zaurrov D, Zappi E. 1996. Novel microbial technologies for the enhancement of plant growth and biocontrol of fungal diseases in crops. *Cah Opt Med* 31:447–456.
- Lefort F, Crovadore J, Gilodi R, Calmin F, Oszako T, Nowakowska JA. 2009. An unknown tree die back caused by *Pseudomonas* species in Switzerland. *Folia For Pol Ser A For* 51:171–175. <https://doi.org/10.5281/zenodo.30753>.
- Lefort F, Douglas GC. 1999. An efficient micro-method of DNA isolation from mature leaves of four hardwood tree species *Acer*, *Fraxinus*, *Prunus* and *Quercus*. *Ann Sci* 56:259–263. <https://doi.org/10.1051/forest:19990308>.
- Andrews S. 2010. FastQC: a quality control tool for high throughput sequence data. <http://www.bioinformatics.babraham.ac.uk/projects/fastqc>.
- Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, Kulikov AS, Lesin VM, Nikolenko SI, Pham S, Pribelski AD, Pyshkin AV, Sirotkin AV, Vyahhi N, Tesler G, Alekseyev MA, Pevzner PA. 2012. SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. *J Comput Biol* 19:455–477. <https://doi.org/10.1089/cmb.2012.0021>.
- Hall TA. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symp Ser* 41:95–98.
- Gurevich A, Saveliev V, Vyahhi N, Tesler G. 2013. QUASt: quality assessment tool for genome assemblies. *Bioinformatics* 29:1072–1075. <https://doi.org/10.1093/bioinformatics/btt086>.
- Tatusova T, DiCuccio M, Badretdin A, Chetvernin V, Nawrocki EP, Zaslavsky L, Lomsadze A, Pruitt KD, Borodovsky M, Ostell J. 2016. NCBI Prokaryotic Genome Annotation Pipeline. *Nucleic Acids Res* 44:6614–6624. <https://doi.org/10.1093/nar/gkw569>.
- Aziz RK, Bartels D, Best AA, DeJongh M, Disz T, Edwards RA, Formsma K, Gerdes S, Glass EM, Kubal M, Meyer F, Olsen GJ, Olson R, Osterman AL, Overbeek RA, McNeil LK, Paarmann D, Paczian T, Parrello B, Pusch GD, Reich C, Stevens R, Vassieva O, Vonstein V, Wilke A, Zagnitko O. 2008. The RAST server: Rapid Annotations using Subsystems Technology. *BMC Genomics* 9:75–90. <https://doi.org/10.1186/1471-2164-9-75>.
- Carattoli A, Zankari E, García-Fernández A, Voldby Larsen M, Lund O, Villa L, Møller Aarestrup F, Hasman H. 2014. In silico detection and typing of plasmids using PlasmidFinder and plasmid multilocus sequence typing. *Antimicrob Agents Chemother* 58:3895–3903. <https://doi.org/10.1128/AAC.02412-14>.
- Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. 1990. Basic local alignment search tool. *J Mol Biol* 215:403–410. [https://doi.org/10.1016/S0022-2836\(05\)80360-2](https://doi.org/10.1016/S0022-2836(05)80360-2).
- Sharma R, Chandel S, Chauhan A, Shirkot CK. 2015. Enhanced phosphorus solubilization by *Bacillus licheniformis* CKA1 using central composite design and response surface methodology. *J Pure Appl Microbiol* 9:3131–3141.